

Atty Dkt No. 9000-0030.10  
USSN: 09/234,733  
PATENT

Accompanying Documents

Accompanying this response are the following documents:

1. Marked-up copy of the claims, showing the amendments made herein (Appendix A);
2. Copy of the currently pending claims, incorporating the amendments made herein (Appendix B);
3. Sequence Listing for the contiguous amino acid sequence shown at positions 29 through 259 of Figures 4A-4C; and
4. Copy of Brenner, Chothia, and Hubbard (1998) *Proc. Natl. Acad. Sci. USA* 95: 6073-6078.

I. AMENDMENTS

In the Specification:

Please amend the specification at page 18, lines 20-35, as follows:

As shown in Figures 4A-4C, the *S. uberis* CAMP factor gene encodes a preprotein of about 256 amino acids (amino acid residues 1 through 256, inclusive, of Figures 4A-4C) that includes an N-terminal signal sequence approximately 28 amino acids in length. The precursor molecule has a calculated molecular weight of 28,363 Da. The mature *S. uberis* CAMP factor thus includes amino acid residues 29 through 256 (SEQ ID NO:5), inclusive, as depicted in Figures 4A-4C. As discussed further below, the portion of the CAMP factor gene encoding the signal sequence can be included in constructs that encode the CAMP factor to direct secretion of the CAMP factor upon expression. Additionally, the CAMP factor signal sequence and the nucleic acid sequence encoding the same can be used with heterologous proteins and nucleic acid molecules, to aid in the secretion thereof.

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Please amend the specification at page 34, lines 18-31, as follows:

A deposit of biologically pure cultures of the following strains was made with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, under the provisions of the Budapest Treaty. The accession number indicated was assigned after successful viability testing, and the requisite fees were paid. The designated deposits will be maintained for a period of thirty (30) years from the date of deposit, or for five (5) years after the last request for the deposit, whichever is longer. Should a culture become nonviable or be inadvertently destroyed, or, in the case of plasmid-containing strains, lose its plasmid, it will be replaced with a viable culture(s) of the same taxonomic description.

Please amend the specification at page 43, lines 20-29, as follows:

*S. uberis* CAMP factor, encoded by pGH-CAMP, was prepared from inclusion bodies as described in Example 1. The antigen was formulated in VSA3 adjuvant which is a combination of EMULSIGEN PLUS from MVP Laboratories, Ralston, Nebraska and Dimethyldioctadecyl ammonium bromide (DDA) from Kodak (Rochester, NY). The final concentration was 25 µg per ml of CAMP factor, 30% EMULSIGEN PLUS, 0.9% TWEEN-80, and 2.5 mg per ml of DDA. The dose volume was 2 cc containing 50 µg of recombinant antigen.

Please amend the specification at page 45, line 11 to page 46, line 2, as follows:

Total Ig titers for CAMP factor were determined by an indirect ELISA. Immulon-2 plates were coated with antigen in carbonate buffer. Prior to use, the plates were blocked with TBST (100 mM Tris Cl, pH 8.0; 150 mM NaCl; 0.05% TWEEN-20) and 3% BSA for 1 hour. After blocking, the plates were washed with distilled water. Serum and milk samples were serially diluted in 3-fold increments using TBST containing 1% BSA. Rabbit antisera for *S. uberis* CAMP factor was also diluted and served as a positive control. Negative control samples contained TBST with 1% BSA.

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The diluted samples and controls were transferred to the coated plates and were incubated for 1 hour at room temperature. The plates were washed thoroughly with distilled water and all wells were incubated with a horse radish peroxidase conjugate of goat anti-IgG diluted 1:2000 in TBST containing 1% BSA. Following a 1 hour incubation at room temperature, the plates were washed with distilled water. The amount of antibody present in samples was visualized using ABT substrate. The titers of each sample were based on the absorbance reading at 405 nm with a reference wavelength of 495 nm. A positive reading for samples was one in which the absorbance was two times the absorbance of the blank (negative control). Titers were determined by taking the reciprocal of the last dilution giving a positive reading. Consistency among assay plates was monitored by the absorbance reading of positive controls.

In the Claims:

Please amend claims 1-6 and 44-46 as follows:

1. (Four times amended) An isolated nucleic acid molecule consisting of a sequence selected from the group consisting of: (a) a sequence encoding an immunogenic polypeptide having at least 90% sequence identity to the contiguous amino acid sequence of SEQ ID NO:2; and (b) a sequence encoding an immunogenic polypeptide having at least 90% sequence identity to the contiguous amino acid sequence of SEQ ID NO:5.
2. (Five times amended) The nucleic acid molecule of claim 1 wherein said nucleic acid molecule encodes an immunogenic polypeptide having a sequence with at least 90% sequence identity to the contiguous amino acid sequence of SEQ ID NO:2.
3. (Four times amended) The nucleic acid molecule of claim 1 wherein said nucleic acid molecule encodes an immunogenic polypeptide having a sequence with at least 90% sequence identity to the contiguous amino acid sequence of SEQ ID NO:5.